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Antimicrobial and Hepatoprotective Potential of *Pterospermum acerifollium* leaves Extracts on *Swiss albino* mice

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ABSTRACT

The objective of present study to investigate the hepatoprotective activity of hydro-alcohollic extract leaves of *Pterospermum acerifollium* against antitubercular drug induced liver damage in swiss albino mice and also performs antimicrobial activity by disc diffusion assay. Successive extractions was performed with different organic solvents viz; hydroalcohollic by cold maceration. The extract was analysed as antioxidant activity as a content of Total phenolic content, Total flavanoid content, Reducing power assay and DPPH Scavenging assay. Antimicrobial activity of methanolic extract was estimated by Agar well diffusion method. Antitubercular drug induced is used as toxicants in hepatoprotective studies in acute condition was analysed by serum biochemical estimations by AST, ALT, ALP and Total Bilirubin. *In-vivo* Antioxidant activity was performed by LPO, GSH, SOD and Catalase. During the collection of tissue for biochemical estimation piece of tissue cut and transferred for Histopathological estimation. The levels were measured and it indicated that the extract had significant antioxidant activity however the results obtained were dose dependent the higher the dose (400 mg/kg) the better activity. The extract administered at dose 400 mg/kg showed better activity. The treatment with hydroalcohollic extract of *Pterospermum acerifillium* reduced the elevated levels of SGOT, SGPT, ALP, TB and also reversed the hepatic damage towards normal which further supports the hepatoprotective activity.

Keywords: Succesive extraction, *In-vivo*, Serum biochemical, Cold maceration

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INTRODUCTION

Herbal derived remedies need a powerful and deep assessment of their pharmacological qualities and safety that actually can be realized by new biologic technologies like pharmacogenomic, metabolomic and microarray methodology. Because of the large and growing use of natural derived substances in all over the world, it is not wise to rely also on the tradition or supposed millenarian beliefs; explanatory and pragmatic studies are useful and should be considered complementary in the acquisition of reliable data both for heal. Toxin or Poison can harm humans or animals. Acute toxicity involves harmful effects in an organism through a single or short-term exposure. Sub-chronic toxicity is the ability of a toxic substance to cause effects for more than one year but less than the lifetime of the exposed organism. Chronic toxicity is the ability of a substance or mixture of substances to cause harmful effects over an extended period, usually upon repeated or continuous exposure, sometimes lasting for the entire life of the exposed organism thcaregiver and patients. The liver is the largest solid organ in the upper abdomen that aids in digestion and removes waste products and worn out cells from the blood. It is considered to be one of the most vital organs that functions as centre of metabolism of nutrients such as carbohydrates, Proteins and lipids and excretion of waste metabolites.

Hepatotoxicity means damage to the liver caused by drugs and other factors resulting in problems in its functioning. Chemicals or drugs that cause hepatotoxicity are called hepatotoxins. Drug induced hepatotoxicity is one of the major problem associated with side effect of drugs. Isoniazid and Rifampicin combination is first line drug for treatment of tuberculosis. Whenever chronic use these drug is present, chances of hepatotoxicity can't be omitted. Mechanism of both drug induced hepatotoxicity is associated with generation of free radicals mediated by their metabolites. In

present plant and plant part is selected on the basis of chances of good antioxidant potential.

MATERIAL AND METHOD

Plant Material:

Plant and plant parts was selected on the basis of Ethano-botanical survey. On the basis of Pharmacological investigation reported on *Pterospermum acerifollium*, it was observed that it has the property to show immunomodulatory potential, Anti-microbial activity, Anti-inflammatory activity, Anti-fertility activity, Anti-depressant activity. However, the plant was reported to possess good quantity of Flavonoid and Phenol.Thus,the leaves of P. acerifollium was selected for the present investigation. The authentication of plant was done by the botanist. A herbarium of plants were submitted to the specimen library of safia college of arts and science, peer gate Bhopal and authenticated by Dr. Zia-Ul-Hassan, Professor and head of department of Botany, safia college of arts and science, peer gate Bhopal. The specimen voucher no. of *Pterospermum acrifollium* Authentication Voucher Specimen No. 304/Bot/Safia/11.[1]

Preparation of plant extract:

The plant were cleaned properly and washed with distilled water to remove any kind of dust particles. Cleaned plants were coarsely powdered in hand grinder. Powdered plants were weighed (250g). Extraction will be performed by maceration using hydroalcohol as solvent in the ratio 3:7.after 7 days it was filtered and the filtrate were kept on water bath 37^0c for 5 days.

Phytochemical Analysis

The Quantitative and Qualitative phytochemical investigation was done for phenolics and flavonoids. Total phenolic content and total flavonoid content was calculated in both the extracts. The results obtained were tested, gallic acid was used as standard for phenolic compounds. Total flavonoid content (TFC) was calculated using rutin as standard.[2]Antioxidants were also detected for their presence in the extracts using ascorbic acid as standard through DPPH assay and reducing power assay.[3]

In-vitro antimicrobial activity

In-vitro antimicrobial activity was also calculated in order to estimate the antimicrobial activity of the extracts. The method chosen to analyse the antimicrobial activity was well diffusion assay.[4]

In-vivo Experiment

Isoniazide + Rifampicin induced Hepatotoxicity

Swiss albino mice weighing between 25-50g were 4 grouped, each group containing of 5 animals. Food was withdrawn 16 hrs before Isoniazide administration to enhance the acute liver toxicity. Group 2, 3, 4 were treated with Isoniazide (75mg/kg, orally) & Rifampicin(150mg/kg, orally) diluted with distilled water was administered on 7^{th} day and after 1 hrs of silymarine treatment and after 1 hrs of extracts treatment and sacrificed 6 hrs after administration of Isoniazide. Silymarine is used as a standard drug. After 6 hrs all the animal were anesthetized using anesthetic ether and blood sample were collected by retro orbital puncture method and serum was used for estimation of AST, ALT, ALP and bilirubin. Immediately afte the collection of blood, the animals was euthanized with over dose of ether, their livers removed, washed in saline and the wet liver volumes were determined. The liver was washed by normal saline, blotted with filter paper and weighed immediately. The livers were preserved in 10% formalin for histopathological studies.[5]

Treatment Schedule

Group 1 – Normal control: The animal received distilled water for 7 days.

Group 2 – Induction of hepatotoxicity by using INH: The animal received distilled water for 7 days and given INH single dose 75mg/kg BW and RIF 150mg/kg on day 8

Group 3 – Standard group: Pretreatment with silymarin 100mg/kg for 7 days followed by a single dose of INH on day 8

Group 4 – Extract group: Pretreatment with plant extracts 200mg/kg for 7 days followed by a single dose of INH on day 8.Blood samples were collected by retro orbital puncture. The blood is collected in dry eppendroff tube. The blood was allowed to coagulate and then centrifuged at 4000 rpm for 15 minutes. The serum was separated and used for biochemical analysis. The animals (n = 6) of each group were sacrificed for determine antioxidant parameters. The liver was excised immediately and used for estimation of antioxidants and thiobarbituric acid reactive substance.[6]

Biochemical estimation:

Blood samples were centrifuged for 10 min at 3000 rpm to separate the serum. AST, ALT, ALP, Total Bilirubin levels were estimated from the serum by using standard kits.

Biostatical interpretation

All data are presented in Mean ±SD. Data were analyzed by One Way ANOVA followed by Benfeffoni's test. $P<0.05$ was considered as level of significance (n=4).

RESULTS AND DISCUSSION

Pterospermum acerifollium leaves were extracted in methanol. The extracts were then tested for several phytochemical constituents. The constituents were carbohydrates, proteins, amino acids, glycosides, alkaloids, saponins, flavonoids, triterpenoids, steroids, tannin, phenolics.

Quantitative phytochemical investigation was done for phenolics and flavonoids. Total phenolic content and total flavonoid content was calculated in both the extracts. The results obtained were tested, gallic acid was used as standard for phenolic compounds. A dilution of both the extract was prepared at 100 µg/ml concentration. The total phenolic content in methanolic extract with respect to gallic acid was found to be 0.051 mg/g equivalent to gallic acid. Total flavonoid content (TFC) was calculated using rutin as standard. The TFC was found to be 0.005 mg/g equivalent to rutin in the methanolic extract. Antioxidants were also detected for their presence in the extracts using ascorbic acid as standard through DPPH assay and reducing power assay. The DPPH assay is performed by making dilutions ranging from concentrations 20, 40, 60, 80 and 100 µg/ml. % inhibition was calculated in order to evaluate the reducing ability of the extract. The methanolic extract was tested and the results obtained were obtained showing % inhibition from 40.33557 % of 20 µg/ml to 43.02013 % of 100 µg/ml indicating significant antioxidant effect.

In vitro Antimicrobial:

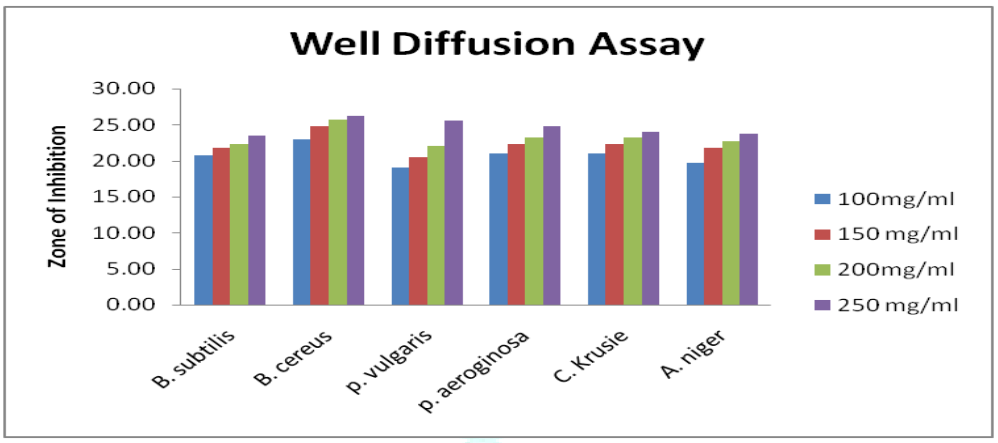
Well diffusion Assay: In-vitro antimicrobial activity was also calculated in order to estimate the antimicrobial activity of the extracts. The method chosen to analyse the antimicrobial activity was well diffusion assay.The agar well diffusion method technique (Bauer *et al.,* 1966) was used to

determine the antibacterial activity of the plant extracts. Inoculation was done on sterile nutrient agar media plate. A sterile 5mm cork borer was used to punch holes after solidification of media. The wells formed were filled with different concentrations of the extract which were labelled accordingly; 100mg/ml, 150mg/ml, 200mg/ml, 250mg/ml. The plates were then left on the bench for 1 hour for adequate diffusion of the extracts and incubated at 37°C for 48 hours in upright condition. The Experiment was repeated triplets and the mean values were calculated.

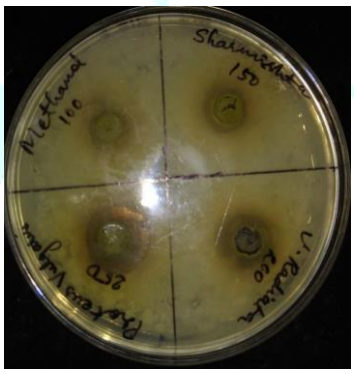
Interpretation of well diffusion assay: After incubation, the diameter of the zones of inhibition around each well were measured to the nearest millimetres along two axis i.e. 90° to each other and the mean of the four reading were then calculated included 5mm well.

Table 1: *In-vitro* Anti-microbial activity (well diffusion method) of extract against various bacterial and fungal strain

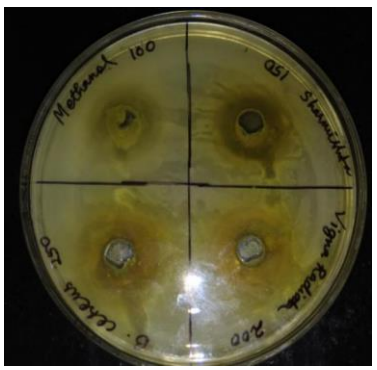
Extract (Hydroalcholic)				
Organisms	**100 mg/ml**	**150 mg/ml**	**200 mg/ml**	**250 mg/ml**
B. subtilis	20.75±0.500	21.75±0.500	22.25±0.500	23.50±1.000
B. cereus	23.00±0.000	24.75±0.500	25.75±0.500	26.25±0.500
P. vulgaris	19.00±0.000	20.50±1.000	22.00±0.000	25.50±0.577
P. aeruginosa	21.00±0.000	22.25±0.500	23.25±0.500	24.75±0.500
C. Krusie	21.00±0.816	22.25±0.957	23.25±0.500	24.00±0.000
A. niger	19.75±0.500	21.75±0.500	22.75±0.500	23.75±0.500



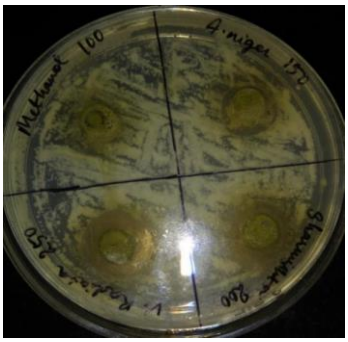
B. subtilis



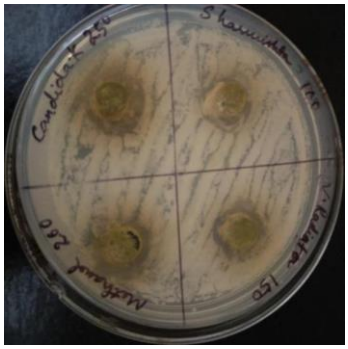
P. vulgaris



C. Krusie



A. niger



P. aeruginosa

B. cereus

Figure 1: Well Diffusion assay of various samples

Antioxidant activity where the values were comparable to that of the standard. Acute oral toxicity was calculated by taking three mice in one group, three groups were taken. Doses tested were 5 mg/kg, 300 mg/kg and 2000 mg/kg. No visible signs or mortality were recorded of any toxicity in the body of the mice.*In-vivo* antioxidant activity was measured through a set of enzymes including LPO, GSH and SOD, SGOT, SGPT, ALP and bilirubin. The levels were measured and it indicated that the extract had significant antioxidant activity however the results obtained were dose dependent the higher the dose (400 mg/kg) the better activity. The extract administered at dose 400 mg/kg showed better activity. To understand the effect of the extract on liver, histology of liver was performed.[9]

Table 2: Effect of the Extract on biochemical parameters (Isoniazide+ Rifampicin)

Treatment Group	Bioassay parameters**			
	SGOT	SGPT	ALP	BILIRUBIN
Vehicle	45.49±1.396	52.52±1.712	18.47±1.245	1.914±0.1802
INH+RIF	136.66±5.68[a]	132.66±2.58[a]	302.33±9.87[a]	1.81±0.111[a]
Silymarin	47.16±3.76 [ab]	38.5±8.36[ab]	182.16±11.7[ab]	0.808±0.056[ab]
S_1(400mg/kg)+INH+RIF	79.833±6.67[ab]	64.5±5.08[ab]	214.16±11.9[ab]	0.893±0.048[ab]

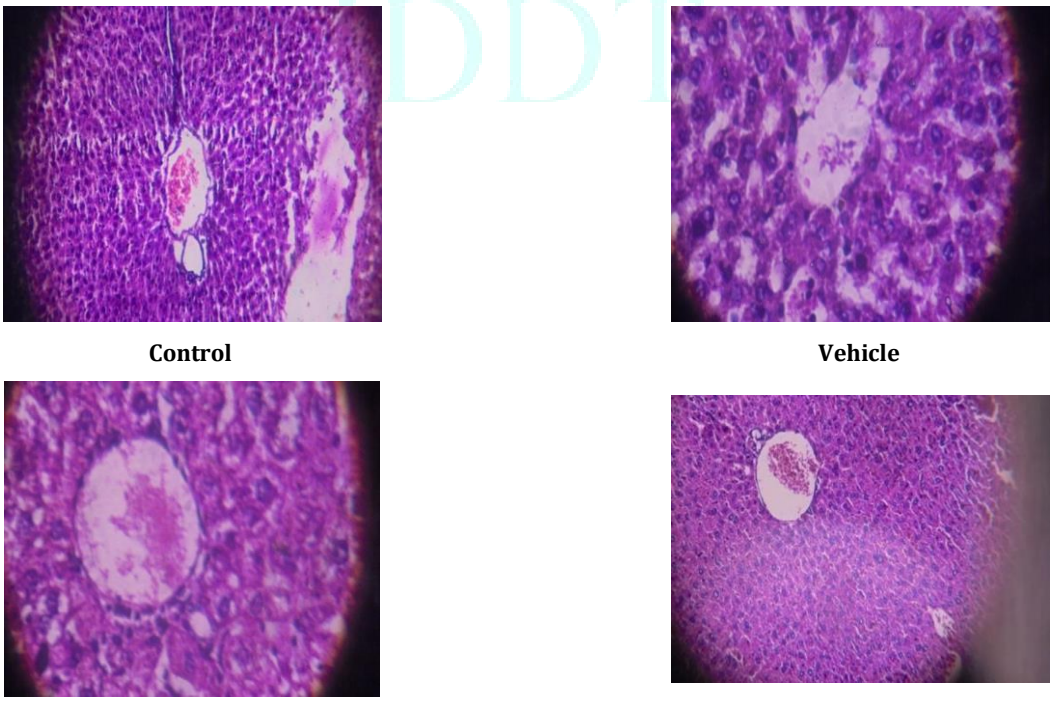
All data are presented in mean ± SD (n =6) a- P<0.05 as compared to vehicle treated group b- P<0.05 as compared to Vehicle + INH+RIF group

Table 3: Effect of the Extract on lipid peroxidation and antioxidant parameters (INH+RIF)

Treatment Group	Bioassay parameters**			
	LPO	GSH	SOD	CATALASE
Vehicle	3.75±0.843	12.13±0.573	37.70±2.075	21.21±0.724
INH	1.25±0.045[a]	4.72±0.0989[a]	5.47±0.329[a]	78.66±12.69[a]
Silymarin	0.618±0.057 [ab]	7.8±0.547[ab]	7.96±0.47[ab]	139.16±5.70[ab]
S_1(400mg/kg)+INH+RIF	0.84±0.0554[ab]	6.95±0.6883[ab]	7.51±0.445[ab]	111.5±7.17[ab]

ll data are presented in mean ± SD (n =6) a- P<0.05 as compared to vehicle treated group b- P<0.05 as compared to Vehicle + INH+RIF group

Histology of Liver:



Control **Vehicle**

Extract 200 mg/kg **Extract 400 mg/kg**

Figure 2: Histology of Liver:

CONCLUSION

In the present study, leaves of *Pterospermum acerifollium* were evaluated for its pharmacognostical, phytochemical and pharmacological aspects.In vitro Antioxidant activity gives better activity in 400mg/kg. Antimicrobial activity was performed and to analyzed with diffusion assay method .The extract was administered to give organisms shows zone of inhibition in dose dependent.The results obtained indicated dose dependent action. The higher the dose, the better the activity. *In vivo* toxicity and serum biochemical along with antioxidant of an enzyme value shows in dose dependent manner. Thus the extract administered at dose 400 mg/kg showed better activity.

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